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Characterization and Incidence of Pigment Production by Human Clinical Group B Streptococci

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Pigment was produced in stab cultures by 97% of 297 group B streptococci isolated from human clinical specimens. The pigment, which was associated with a membranous cell fraction, showed a four-banded absorption spectrum similar to that of a carotenoid, with maxima at 435, 566, 485, and 525 nm. Addition of glucose to the growth medium suppressed pigment production in most strains. Only 37% of strains from bovine sources produced pigment.

Group B streptococci (*Streptococcus agalactiae*) have been recognized as a significant cause of neonatal and adult disease (1, 2, 10, 15). Although these organisms are classically identified by serological analysis (3, 9), presumptive identification is often based on hippurate hydrolysis (5) or the CAMP test (4, 14). Various recent studies have shown that 85 to 98% of human group B streptococci produce pigment when grown anaerobically on certain media (6–8, 10–12). We have introduced a more convenient and reliable method for detecting pigment production, with the use of a stab culture in a modified medium (11, 12). In the present investigation, we have further evaluated the stab technique in a larger number of newly isolated group B streptococci from various clinical sources. Since the chemical nature of the group B pigment has not been described previously, we have also studied this and other aspects of pigment production.

Streptococci were tested for pigment production and confirmed as group B by hippurate hydrolysis and serological grouping as previously described (12). Streptococci were typed using antiserum kindly provided by Hazel Wilkinson of the Center for Disease Control (Atlanta, Ga.). To prepare cells for pigment characterization, a clinical isolate of serological type II was grown in 10 liters of DMS broth (11) at 37°C for 72 h in a stationary flask filled nearly to the neck. After centrifugation, the cell pack, which contained all of the red-orange pigment, was washed with 200 ml of 0.15 M NaCl. Small portions of these cells were removed and suspended in various solvents to determine the extractability of the pigment as described below. For preparation of membrane fragments, the cell pack was suspended in an equal volume of 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.6, and disrupted by sonic oscillation for eight 20-s intervals with intermittent cooling (0°C) in a Branson Sonifier at power setting 4 (Branson Instruments, Inc., Danbury, Conn.). The sonically treated material was centrifuged at 12,000 × *g* for 7 min. The supernatant fraction was removed and subjected to ultracentrifugation (90,000 × *g*) for 2 h in a Spinco model L ultracentrifuge at 4°C.

Incidence of pigment production. In a 12-month period, 297 group B streptococci were isolated from the following sources: urine (94), cervix and vagina (78), wounds (27), blood (6), ear (6), autopsy lung (4), breast (94), and sputum and throat (44). (Group B streptococci were not routinely sought in throat specimens.) Of the 297 organisms serologically identified as group B, 290 (97%) produced pigment in stab cultures, and 297 (100%) hydrolyzed hippurate. A few strains exhibited only a faint pigment which could only be detected by examination in direct sunlight. Pigment production was not observed with any other groups of streptococci or with any other organisms from our clinical isolates.

Effect of serological type. Representatives of all types produced pigment. Of the seven non-pigment producers we isolated, two were type Ib, one was type Ia, one was type II, and three were nontypable. All of the type Ic and III strains were pigmented. Six of the non-pigment producers were from the upper respiratory tract, and one was from urine (female). All organisms from neonatal and vaginal and cervical specimens were pigmented.

Pigment in bovine isolates. Forty strains isolated from cases of bovine mastitis were obtained from N. Norcross, College of Veterinary Medicine, Cornell University Ithaca, N.Y., and from R. Brown, National Animal Disease Laboratory, Ames, Iowa. Only 37% of these produced

pigment, confirming other reports that pigment production is an unreliable characteristic of animal strains (10). Repeated subculture did not alter the production of pigment by the pigment producers or the non-pigment producers.

Effect of glucose on pigment production. Todd-Hewitt medium, which is widely used for growing streptococci, will not reliably support the production of pigment by most group B streptococci. The major difference between DMS (11) and Todd-Hewitt media is the 0.2% glucose in Todd-Hewitt. Thus, DMS agar was prepared with different concentrations of glucose, and 72 consecutive pigmented organisms isolated from different individuals were tested. Pigment production was suppressed by 0.1% glucose with a few isolates, whereas 0.2% glucose suppressed pigment in 74% of these isolates. No isolate formed pigment in 0.4% glucose. The effect of glucose was not related to the serological type of the organism.

Characterization of the pigment. The pigment was not extractable from intact cells with water, methanol, ethanol, acetone, diethyl ether, petroleum ether, alcoholic KOH, or mixtures of ether and acetone or ethanol and acetone when tested at room temperature or at 56°C. The pigment was extracted from cells by phenol or phenol-glycerol (3:1, by weight) at room temperature.

To determine pigment localization, intact cells were sonically disrupted and centrifuged at 12,000 × *g*, and the layering in the centrifuge tube was examined. Above the bottom layer of reddish-brown undisrupted cells was a substantial tomato-red, gelatinous layer, presumably large pieces of cell wall plus membranous fragments. The supernatant solution was light red in color and slightly turbid. When this was subjected to ultracentrifugation (90,000 × *g*) for 2 h, the red color was completely sedimented into a tomato-red pellet consisting of small membrane fragments. The absorption spectrum obtained by dispersing a small portion of the membrane fraction in water revealed four peaks in the region between 400 and 550 nm (Fig. 1). The same spectra were obtained with phenol-glycerol extracts of whole cells. The shape of these peaks of the absorption curve in this region was similar to that exhibited by various carotenoids. For comparison, the spectrum of α-carotene (Sigma Chemical Co., St. Louis, Mo.) dissolved in phenol is shown. The group B pigment had peaks at 435, 455, 485, and 525 nm, clearly distinguishing it from α-carotene. These absorption peaks also differentiated the group B pigment from the carotenoid pigment formed by a few strains of *S. faecium* (13). In addition, the latter

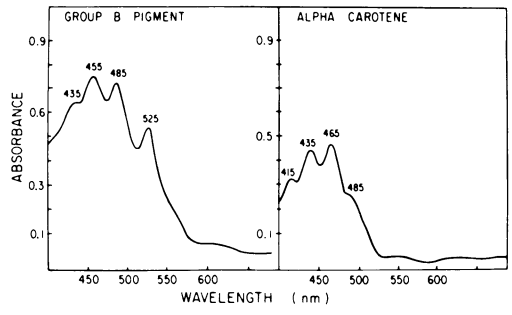

FIG. 1. *Spectra of pigment extracted from group B streptococcus as compared with α-carotene. A small amount of the membranous fraction from the sonically disrupted cells (prepared as described in the text) was dispersed in water to give a solution suitably dilute for spectroscopy. For comparison, a small amount of crystalline α-carotene was dissolved in phenol. Spectra were scanned with a Cary 14 automatic recording spectrophotometer.*

pigment is best produced during aerobic growth, whereas the group B pigment was only formed anaerobically. These observations suggest that the group B pigment may be a carotenoid which is not a soluble component in the cell cytoplasm, but is localized in a membranous fraction of the cell. However, further studies are needed to completely establish the chemical nature of this pigment.

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